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## New county records of *Aedes aegypti* and *Aedes epactius* in Colorado

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### Abstract

In August and September, 2017 we conducted mosquito surveillance in southeastern Colorado by using ovitraps and larval sampling. The aim was to determine if there were established populations of *Aedes aegypti* and *Ae. albopictus* in the region. A single female *Ae. aegypti* was reared from eggs collected in La Junta CO, but *Ae. albopictus* was not detected. Three other species were reared from eggs and/or larvae; *Ae. epactius*, *Culex restuans* and *Cx. pipiens*. *Aedes aegypti* and *Ae. epactius* were detected for the first time in Otero and Baca counties respectively and these are new county records for Colorado. Both species were detected in very low numbers suggesting extremely low population density or sporadic introductions into southeastern Colorado.

### Keywords

*Aedes aegypti*; *Aedes epactius*; Colorado

The yellow fever mosquito, *Aedes (Stegomyia) aegypti* (L), is an invasive species that has been in the United States for centuries (Nelson 1986). *Aedes aegypti* is a vector of several arboviruses of public health importance including yellow fever virus (Reed et al. 1900), dengue viruses (Rudnick 1965), chikungunya virus (Moore et al. 1974) and Zika virus (Marchette et al. 1969). It is believed to have originated in Africa (Tabachnick 1991), and currently has a worldwide distribution in the tropics and sub-tropics. Occasionally *Ae. aegypti* populations have been detected in temperate zones in North America. In the United States *Ae. aegypti* has been found throughout the southern states north to New Jersey, Illinois Kansas and the southwest including California, Arizona and southern New Mexico (Darsie and Ward 2005, Hahn et al. 2016, 2017). The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* (Skuse), is also an invasive species first detected in the United States in 1985 (Sprenger and Wuithiranyagool 1986). It is a vector of dengue virus (Rudnick and Chan 1965), chikungunya virus (Paupy et al. 2012), Zika virus (Grard et al. 2014) and many other pathogens of public health importance (2004). *Aedes albopictus* is native to Asia (Benedict et al. 2007), it is believed to have been introduced to the United States from Japan through the used tire trade (Hawley et al. 1987). In the United States, *Ae. albopictus* populations have been detected throughout the southern states, the eastern seaboard and some parts of the Midwest (Hahn et al. 2016, 2017).

*Aedes aegypti* is not native to the state of Colorado and prior to this study it had only been detected once in this state; in specimens collected from Pueblo County in southern Colorado in 2010 (Rose et al. 2015) (Figure 1). Established populations of *Ae. aegypti* are not known to exist in Colorado, the detection in Pueblo County in 2010 (Rose et al. 2015) was thought to be an accidental introduction and an isolated incident. However, *Ae. aegypti* is capable of causing dengue outbreaks at relative abundance levels of 0.5 – 1.5 pupae/person (Focks et al. 2000), therefore even modest introductions or low population levels may pose a public health threat. Similarly, *Ae. albopictus* is not native to Colorado, however, for several years, a population have been detected in Larimer County in northern Colorado (Hahn et al. 2016, 2017) suggesting that Colorado can support populations of *Ae. albopictus*. Surveillance for *Ae. aegypti* and *Ae. albopictus* is not routinely conducted in Colorado and therefore there is the possibility of undetected introductions or even established low level populations in the southern part of the state. Currently, routine mosquito surveillance in Colorado primarily focuses on *Culex* vectors of WNV especially *Cx. pipiens* and *Cx. tarsalis* (Fauver et al. 2016). There is therefore the need to find out if there are established populations of *Ae. aegypti* and *Ae. albopictus* in Colorado, especially in the southern part of the state to evaluate the risk of transmission of *Ae. aegypti* and *Ae. albopictus* borne arboviruses in the state.

We placed ovitraps in seven cities in southeastern Colorado, (Pueblo, Walsenburg, Trinidad, La Junta, Las Animas, Lamar and Springfield). Sampling efforts varied from city to city depending on availability of suitable sampling sites. In each city, we selected sites that were more likely to support populations of *Ae. aegypti* and *Ae. albopictus*; junk yards, tire shops, abandoned houses, railroad yards and container cluttered homes. We placed 6 ovitraps in the proximity of each sampling site. In Pueblo we sampled 2 sites (12 ovitraps), in Walsenburg one site (6 ovitraps), in Trinidad one site (6 ovitraps), in La Junta three sites (18 ovitraps), in Las Animas one site (6 ovitraps), in Lamar two sites (12 ovitraps) and in Springfield one site (6 ovitraps). Overall, we used 66 ovitraps and the same sites were samples in both August and September 2017. Ovitrap were water-filled 22 Oz black plastic cups (Giacona Container Corporation, Jefferson, LA) with seed germination paper (Anchor Paper Company, St Paul, MN) as the oviposition substrates. Ovitrap were placed in the field on August 11 and September 8, and collected from the field on August 18 and September 15 respectively. In addition, we collected larvae from discarded containers and from the ovitraps by using turkey basters and white plastic trays. The larvae were placed in plastic 4 oz Whirl-Pak bags (Cole-Palmer, Vernon Hills, IL) and transported to the Centers for Disease Control and Prevention (CDC) laboratory in Fort Collins, CO, where they were reared to adult stage and identified to species using the taxonomic keys of Darsie & Ward (2005). Larvae were reared in the water in which they were collected (tire water) and not fed in the laboratory. The eggs were hatched and reared in tap water and fed on liver powder (MP Biomedicals, LLC, OH). Mosquito rearing was done in Forma Environmental Chambers (Thermo Electron Corporation, Marietta, OH) at 28°C, 80% RH, and 16:8 h LD photoperiod. Pupae were placed in tap water, in 100ml plastic cups in mosquito breeders (BioQuip, Rancho Dominguez, CA). The adult mosquitoes were identified soon after emerging.

We conducted 462 trap nights but we only able to count 470 eggs on substrates from Springfield, CO. There were large amounts of debris on the substrates and that made it difficult to detect and count the mosquito eggs accurately. Despite that, we flooded all substrates without detectable mosquito eggs and one larva emerged from the substrates from La Junta collected in September 2017. We reared the larva to the adult stage and identified it as an *Ae. aegypti* female. We also flooded the substrates from Springfield, but very few of the eggs hatched, we were able to rear two larvae to the adult stage and both were female *Ae. epactius*. In addition, we identified four *Cx. restuans* and forty-two *Cx. pipiens* from larvae collected in Springfield. Overall, we collected four species; one *Ae. aegypti* from La Junta, two *Ae. epactius*, four *Cx. restuans*, and forty-two *Cx. pipiens* from Springfield. The single female *Ae. aegypti* was reared from an egg collected in La Junta is the second detection of *Ae. aegypti* in southern Colorado in seven years, and the first detection of this species in Otero County (Rose et al. 2015, Hahn et al. 2016, 2017). We do not know if there are established populations of *Ae. aegypti* in southern Colorado especially since there is no routine mosquito surveillance in southeastern Colorado, outside of the city of Pueblo; more investigations are needed to address this issue.

We did not detect *Ae. albopictus* in southeastern Colorado and the reason why is not clear at this time. There is the possibility that this species does not exist in southeastern Colorado and the maps published by Hahn et al. (2016, 2017), support this assumption. However, our studies took place over a short period, only 14 days, and there is the possibility that we did not detect *Ae. albopictus* because of this short study duration.

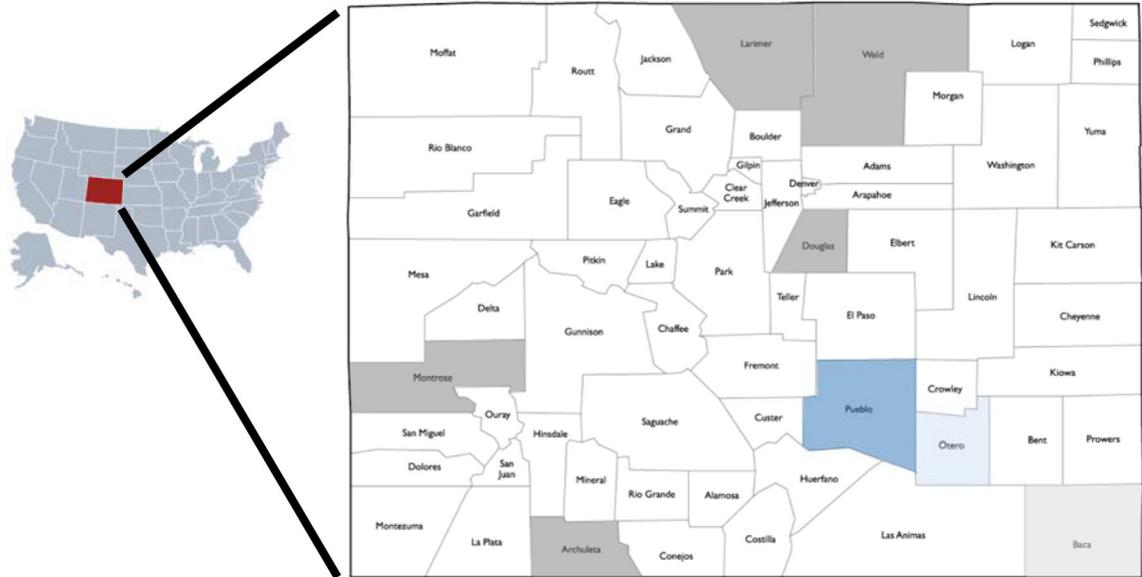
We report the first record of *Aedes (Ochlerotatus) epactius* Dyar and Knab in Baca County and in southeastern Colorado. The specimens were collected as eggs at a tire shop in Springfield, CO. This increases the number of counties in Colorado in which *Ae. epactius* has been detected to six and suggests a much broader distribution of this species in Colorado. The public health importance of *Ae. epactius* is currently not well understood. *Aedes epactius* is a competent vector for Jamestown Canyon Virus (JCV) (Heard et al. 1991), but it has not yet been associated with JCV outbreaks despite the fact that the distribution ranges of JCV and *Ae. epactius* overlap (Calisher 1983, Darsie and Ward 2005). Furthermore, JCV has not yet been detected in field-collected *Ae. epactius*. However, *Ae. epactius* is an aggressive human biter and in some areas it is a nuisance species (Carpenter and LaCasse 1955). *Aedes epactius* is widely distributed in North and Central America (Carpenter and LaCasse 1955, Weissmann 2016), but it is not a common species in the state of Colorado. It was previously collected in two western counties, Archuleta and Montrose, (Harmston and Lawson 1967), and three northern counties Larimer, Weld and Douglas (Rose et al. 2017) (Figure 1).

Our results strongly suggest that routine surveillance for mosquito vectors is needed in southeastern Colorado to understand the true range of *Ae. aegypti*, and to enhance our understanding of the dynamics and seasonality of *Ae. aegypti* and other vector species in this region. In addition to establish if *Ae. albopictus* exists in this part of the state.

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**Figure 1.** The distribution of *Ae. aegypti* and *Ae. epactius* in Colorado after adding in our new records from the fall of 2017. The previous *Ae. aegypti* detection is represented by a darker blue shade (Pueblo County) and the new detection by a lighter blue (Otero County) shade. The previous *Ae. epactius* detections are represented by the darker grey shade (Larimer, Weld, Douglas and Montrose Counties) and new detection by the lighter gray shade (Baca County).